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Chromosomal Dynamics in Platyrrhinae by Mapping Bacs Probes

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Introduction

Molecular cytogenetics by chromosome painting permits to detect, at molecular level, chromosomal homologies and interchromosomal rearrangements occurred during genomes evolution. Chromosome painting does not permit the detection of intrachromosomal rearrangements (inversions, activation of new centromere); those rearrangements, can be detected at molecular level using cloned DNA such as Bacterial Artificial Chromosomes (BACs) (Ventura *et al.*, 2004).
In the present work we selected probes derived from human chromosome 4 because comparative cytogenetic data showed that this synteny is less conserved than previously thought (Picone *et al.*, 2010). At the begins of the cytogenetics era the ortologues to human synteny 4 in Primates has been considered a conserved single submetacentric chromosome (Haig *et al.*, 1999) but by painting approach too many exception have been showed (Dumas, 2011). Stanyon and collegues (2008) by mapping BACs probes showed different centromeres positions in the homologues to human chromosome 4 in New word monkeys and Old word monkeys. We mapped an appropriate human BACs probe set on the homologues of human chromosome 4 in *Saimiri sciureus*, *Saguinus oedipus*, Platyrrhinae, and on *Pongo pygmaeus*, Catarrhinae, used as out-group, with the aim to study fine chromosomal evolution in New Word monkeys.

Materials and Methods

Metaphases from non human primates were obtained from lymphoblast or fibroblast cell lines of: common squirrel monkeys (*Saimiri sciureus*, SSC), cotton-top tamarins (*Saguinus oedipus*, SOE), borneo orangutan (*Pongo pygmaeus pygmaeus*, PPY). The human BAC probes a) RP11-1150b4, b) RP11-637n1, c) RP11-166k6, d) RP11-70L18, e) RP11-443J23 clones were selected on the UCSC and mapped on the platyrrhinae species and on *P. pygmaeus* (Tab. 1).

Standard FISH protocols were applied. Hybridizations were performed in 50% formamide, 10% dextran sulfate, 2 × SSC at 37°C, in the presence of human Cot1 DNA (Gibco-BRL). Post-hybridization washing included 50% formamide, 2 × SSC at 42°C, or 50% formamide, 1 × SSC at 37°C, followed by three washes in 1 × SSC at 42°C. The chromosomes were stained with DAPI (4',6-diamidino-2- phenylindole). Digital images were obtained using a Leica DMRXA2 microscope equipped with a cooled CCD camera (Princeton Instruments) and arranged using Adobe Photoshop software.

Probes	Species
a) RP11-1150b4,	SSC, CJA, SOE, PPY
b) RP11-637n1,	SSC, PPY
c) RP11-166k6,	SSC, PPY
d) RP11-70L18,	SSC, SOE, PPY
e) RP11-443J23	SSC, SOE, PPY (CJA –Stanyon et al., 2008)
f) RP11-455K3	(CJA -Stanyon et al., 2008)

Tab.1. *Human BAC probes used in the present work and the list of the species on which they have been mapped.*

Results

All hybridization experiments are repeatable, signals are bright and in agreement with painting data regarding the orthologues of human chromosome 4 in Platyrrhinae (SSC1- Stanyon *et al.*, 2000, SOE7, Neusser *et al.*, 2001). We have mapped BAC probes a), b), c), d) and e) on *S. sciureus* and *P. pygmaeus*. Probe a), e) and d) have been mapped also on *S. oedipus* (Fig.1).
Probe a) maps in a q arm position on chromosome 1 of *S. sciureus*, on chromosome 7 of *S. oedipus*; in p arm position on chromosome 3 of *P. pygmaeus*. Probes b), c), d), and e) map in the p arm on chromosome 1 of *S. sciureus* and in the q arm position of *P. pygmaeus* chromosome 3. Probe e) maps in a p arm position and probe d) in a q arm position on chromosome 7 of *S. oedipus*. The obtained results have been compared with probe e) and f), previously, mapped on *Callithrix jacchus* chromosome 3 (Stanyon *et al.*, 2008).

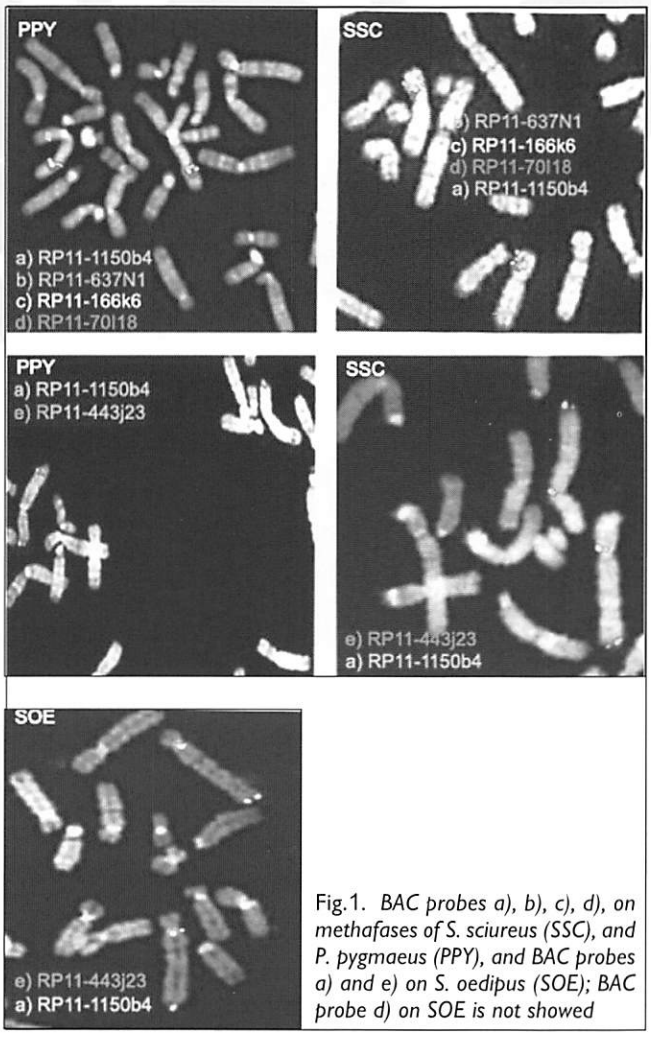

Fig.1. *BAC probes a), b), c), d), on methafases of S. sciureus (SSC), and P. pygmaeus (PPY), and BAC probes a) and e) on S. oedipus (SOE); BAC probe d) on SOE is not showed*

Discussion

Our results permits us to identify, at a high level of resolution, intrachromosomal rearrangements on the homologues of human chromosome 4, not easily detectable by painting. The ortologues of human synteny 4 in Primates has been considered a conserved single submetacentric chromosome (Haig *et al.*, 1999) but many exceptions have been showed (see Dumas, 2011 for a review). By banding analysis Dutrillaux (1979) hypothized a pericentromeric inversion or a centromeric shift occurred on *C. jacchus* synteny 4. Stanyon and collegues (2008) studying chromosomal marker orders in primates showed by BACs mapping a new evolutionary centromere on the homologous of human chromosome 4 in *C. jacchus,* and two more in *L. lagotrica* homologues. Our probes mapping permitted us to show different rearrangements: probes a) falls on opposite position in the species analysed, in a p arm position on chromosome 3 of *P. pygmaeus* and in a q arm position in Platyrrhinae (CJA3, SSC1, SOE7) (Fig. 2).
The apparent different position of the BAC signals, as previously demonstrate is the result of the occurrence of a new evolutionary centromere (between probe e and f) without any change in markers order (Stanyon *et al.*, 2008, Rocchi *et al.,* 2009).
A new rearrangement never showed before is demonstrated considering the mapping of probe b) to e) on *S. sciureus* and previous data present in literature on *C. jacchus* (Fig. 2). Probes b) to e) do not fall (as it is possible to suppose looking *C.jacchus*) in a q arm position but in a p arm position on chromosome 1 of *S. sciureus* with a different orientation allowing us to propose the occurrence of an inversion in *S. sciureus* chromosome 1. Furthermore, the hybridization of BAC probes e) and d) on the homologues of human chromosome 4 in *S. oedipus*, chromosome 7 and in *S. sciureus* permits to show another rearrangement (Fig. 2). Both probes e) and d) map in a p arm position on chromosome 1 of *S. sciureus;* on the contrary those probes map in different arms on chromosome 7 of *S. oedipus,* probe d) falls in a p arm position and e) in a q arm, both close to the centromere. This result shows different centromeric positions in the

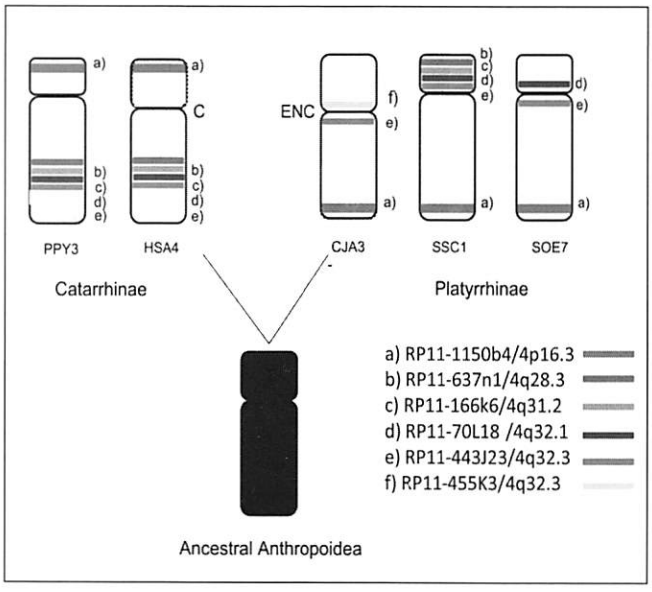

Fig. 2. *BAC probes localization on the homologues of human chromosome 4 in New and Old World monkeys. The apparent different position of probe a) in Platyrrhinae (CJA3, SSC, SOE7) and Catarrhinae (PPY3, HSA4) is the result of a new evolutionary centromere (ENC) formation showed by Stanyon et al., 2008 between probes e) and f). Probe b),c),d), and e) in SSC1 fall, not as it is possible to extrapolate from CJA3 in a q arm position, but in a p arm position as a result of an inversion. Probe e) and d) location on SSC1 and SOE7 show a different centromere position in the two species as result of another inversion or a ENC.*

two species (with the centromere below probe e) in *S. sciureus* and over e) in *S. oedipus* indicating presumably the occurrence, in the ancestor of the species, of the same pericentric inversion, and a successive new centromere formation or a little inversion in *S. oedipus*.

Conclusion

Ours results and data from literature permits to show a high level of intrachromosomal rearrangements in the dynamics of human synteny 4 in Platyrrhinae. Two new evolutionary centromeres have been identify on two of the tree fragments homologues to human synteny *4* in *Lagothrix lagotricha* (Stanyon *et al.*, 2008). Even where human synteny 4 is conserved as a single chromosome

is possible to show intrachromosomal rearrangements: a new evolutionary centromeres has been found in *C. jacchus* chromosome 3 (Stanyon et al., 2008). We show the occurrence of an inversion on the homologues of human chromosome *4* in *S. sciureus* and *S. oedipus*, and the occurrence of a successive, other, little pericentric inversion or a new evolutionary centromeres in *S. oedipus* chromosome 7. More BAC mapping should be performed to better discriminate rearrangements and clarify synteny 4 intrachromosomal dynamics in Platyrrhinae. Through this work is showed the usefulness of the BAC mapping approach in the identification of intrachromosomal rearrangements utilizable as markers in phylogenetic studies.

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